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Evidence for involvement of the insula in the psychotropic effects of THC in humans: a double-blind, randomized pharmacological MRI study

Hendrika H. van Hell

University of Wollongong, erikavh@uow.edu.au

Matthijs G. Bossong

King's College London, University Medical Center Utrecht

Gerry Jager

University Medical Center Utrecht

Gert Kristo

University of Tilburg, University Medical Center Utrecht

Matthias J. P van Osch

Leiden University Medical Center

See next page for additional authors

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Abstract

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Keywords

randomized, pharmacological, evidence, involvement, insula, psychotropic, study, effects, mri, thc, humans, double, blind

Disciplines

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Authors

Hendrika H. van Hell, Matthijs G. Bossong, Gerry Jager, Gert Kristo, Matthias J. P van Osch, Fernando Zelaya, Rene S. Kahn, and Nick F. Ramsey

Evidence for involvement of the insula in the psychotropic effects of THC in humans: a double-blind, randomized pharmacological MRI study

Hendrika H. van Hell¹, Matthijs G. Bossong¹, Gerry Jager^{1,2}, Gert Kristo^{1,3},
Matthias J. P. van Osch⁴, Fernando Zelaya⁵, René S. Kahn⁶ and Nick F. Ramsey¹

¹ Rudolf Magnus Institute of Neuroscience, Department of Neurology and Neurosurgery, University Medical Center Utrecht, Utrecht, The Netherlands

² Division of Human Nutrition, Wageningen University, The Netherlands

³ Department of Medical Psychology, University of Tilburg, The Netherlands

⁴ Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands

⁵ Institute of Psychiatry, King's College London, UK

⁶ Rudolf Magnus Institute of Neuroscience, Department of Psychiatry, University Medical Center Utrecht, Utrecht, The Netherlands

Abstract

The main reason for recreational use of cannabis is the 'high', the primary psychotropic effect of Δ^9 -tetrahydrocannabinol (THC). This psychoactive compound of cannabis induces a range of subjective, physical and mental reactions. The effect on heart rate is pronounced and complicates bloodflow-based neuroimaging of psychotropic effects of THC. In this study we investigated the effects of THC on baseline brain perfusion and activity in association with the induction of 'feeling high'. Twenty-three subjects participated in a pharmacological MRI study, where we applied arterial spin labelling (ASL) to measure perfusion, and resting-state functional MRI to assess blood oxygen level-dependent signal fluctuation as a measure of baseline brain activity. Feeling high was assessed with a visual analogue scale and was compared to the imaging measures. THC increased perfusion in the anterior cingulate cortex, superior frontal cortex, and insula, and reduced perfusion in the post-central and occipital gyrus. Baseline brain activity was altered, indicated by increased amplitude of fluctuations in resting-state functional MRI signal after THC administration in the insula, substantia nigra and cerebellum. Perfusion changes in frontal cortex were negatively correlated with ratings of feeling high, suggesting an interaction between cognitive control and subjective effects of THC. In conclusion, an acute THC challenge altered baseline brain perfusion and activity, especially in frontal brain areas involved in cognitive and emotional processes, and the insula, associated with interoceptive awareness. These changes may represent the THC-induced neurophysiological correlates of feeling high. The alterations in baseline brain perfusion and activity also have relevance for studies on task-related effects of THC on brain function.

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Key words: Arterial spin labelling, fMRI, perfusion, resting-state, THC.

Introduction

Cannabis is one of the most widely used drugs in the world. Its main psychoactive constituent, Δ^9 -tetrahydrocannabinol (THC), produces a number

of acute, dose-dependent psychotropic effects, such as 'feeling high', relaxation and euphoria which are regarded as the main reason why people use cannabis (Green *et al.* 2003). In addition, feeling high has been reported as the most stable subjective effect of THC or cannabis administration, as recently reviewed by Zuurman *et al.* (2009). Effects on cognition are also frequently reported, such as memory impairments (Bhattacharyya *et al.* 2009; Grotenhermen, 2002). In addition to these, THC has prominent effects on blood pressure and heart rate, and causes cerebral

Address for correspondence: Prof. Dr. N. F. Ramsey, Department of Neurology and Neurosurgery, Rudolf Magnus Institute for Neuroscience, University Medical Center Utrecht, Heidelberglaan 100, mail stop G.03.124, 3584 CX Utrecht, The Netherlands.
Tel.: +31-(0)8875556863 Fax: +31-(0)302542100
Email: n.f.ramsey@umcutrecht.nl

vasodilatation (Wagner *et al.* 2001). All of these effects can be expected to reflect changes in brain function and perfusion. Brain-imaging studies have measured acute effects of cannabis (THC) on baseline brain perfusion in humans, using positron emission tomography (PET) (Chang & Chronicle, 2007; Martin-Santos *et al.* 2010), the main outcome of which is that THC administration increases regional cerebral blood flow (rCBF) in prefrontal, insular, and anterior cingulate regions. Changes in rCBF have been associated with many aspects of THC-induced behavioural effects such as a changed time perception, increased anxiety, intoxication levels and arousal (Mathew & Wilson, 1993; Mathew *et al.* 1989, 1992, 1997, 1998, 1999, 2002). Yet, the specific neurophysiological correlate of the main reason for recreational use of THC, the feeling high, remains to be elucidated.

Recent advances in magnetic resonance imaging (MRI) techniques have provided the arterial spin labelling (ASL) technique, which offers a quantitative and non-invasive measure of CBF (Petersen *et al.* 2006). ASL employs blood as an endogenous tracer by magnetically labelling arterial blood water (Detre & Alsop, 1999; Williams *et al.* 1992). This technique can be administered repeatedly within a scan session, making it very attractive for studies involving a pharmacological challenge (Liu & Brown, 2007).

Perfusion, however, does not immediately reflect brain activity when a drug is administered with potential effects on cerebrovasculature, such as THC. An additional measure of brain activity would thus be informative for assessing effects of THC on baseline brain function, the most obvious being functional MRI (fMRI). Both techniques can be applied within the same scan session, allowing for a rich assessment of effects of a pharmacological challenge. Moreover, it constitutes an advancement in study design compared to the previously mentioned PET studies (Mathew & Wilson, 1993; Mathew *et al.* 1989, 1992, 1997, 1998, 1999, 2002) because it allows for a combination of within-subject placebo–drug comparisons (multiple sessions) without exposing subjects to radioactivity twice, with pre- and post-administration measurements for corrections of baseline values, thereby improving internal validity of the data.

Blood oxygen level-dependent (BOLD) fMRI is currently the preferred technique for studying the acute effects of THC on specific brain functions. fMRI studies have reported THC-induced alterations in brain activity during emotional, inhibitory, and memory processes (Bhattacharyya *et al.* 2009; Borgwardt *et al.* 2008; Fusar-Poli *et al.* 2009). These

pharmacological imaging studies used task-dependent fMRI, i.e. fMRI in combination with a cognitive challenge. So far, these have not addressed the effects that THC may have on baseline brain activity during rest. Here we present a pharmacological MRI (phMRI) study aimed at bridging this knowledge gap by investigating the acute psychotropic effects of THC on brain neurophysiology. We combined a randomized, double-blind, placebo-controlled design with ASL and resting-state fMRI techniques to assess the intrinsic effects of THC administration on global and regional perfusion and on BOLD signal fluctuation as a measure of baseline brain activity. Additionally, subjective effects of THC were measured with visual analogue scales (VAS), and heart rate and respiration were continuously monitored. We expected that THC administration, in line with findings from PET studies, would increase brain perfusion in a region-specific manner, predominantly in frontal and limbic areas.

For analysis of the resting-state data we chose to apply a robust measure of signal fluctuations, and not to rely on assumptions in terms of specific fluctuation frequencies [amplitude of low-frequency fluctuations (ALFF); Zou *et al.* 2008] or in terms of specific networks [independent component analysis (ICA); Damoiseaux *et al.* 2006]. A reliable measure of fluctuation amplitude of the BOLD signal over time is the temporal signal-to-noise ratio (tSNR), computed by dividing the mean BOLD signal over a period of time by its standard deviation. The tSNR is first computed per voxel, and then averaged across all voxels in the brain. Given the fact that the distribution of power across frequencies in fMRI follows a 1/frequency relationship (Zarahn *et al.* 1997), the tSNR is dominated by lower frequencies. Hence, a high tSNR corresponds largely to low ALFF and vice versa. As the tSNR is affected by all frequencies, it reflects all physiological fluctuations (Zou *et al.* 2008). Given that an increase in brain activity is accompanied by increased spontaneous signal fluctuations (Yang *et al.* 2007), we hypothesized that the psychotropic effects of THC would be reflected by reduced tSNR in the involved regions.

Materials and methods

This study is part of the Pharmacological Imaging of the Cannabinoid System (PhICS) study, a comprehensive research project on the role of the endocannabinoid system in the regulation of cognitive brain function in healthy volunteers and patients with psychiatric disorders. Methods and study protocol are reported in detail in a methodological paper (van Hell

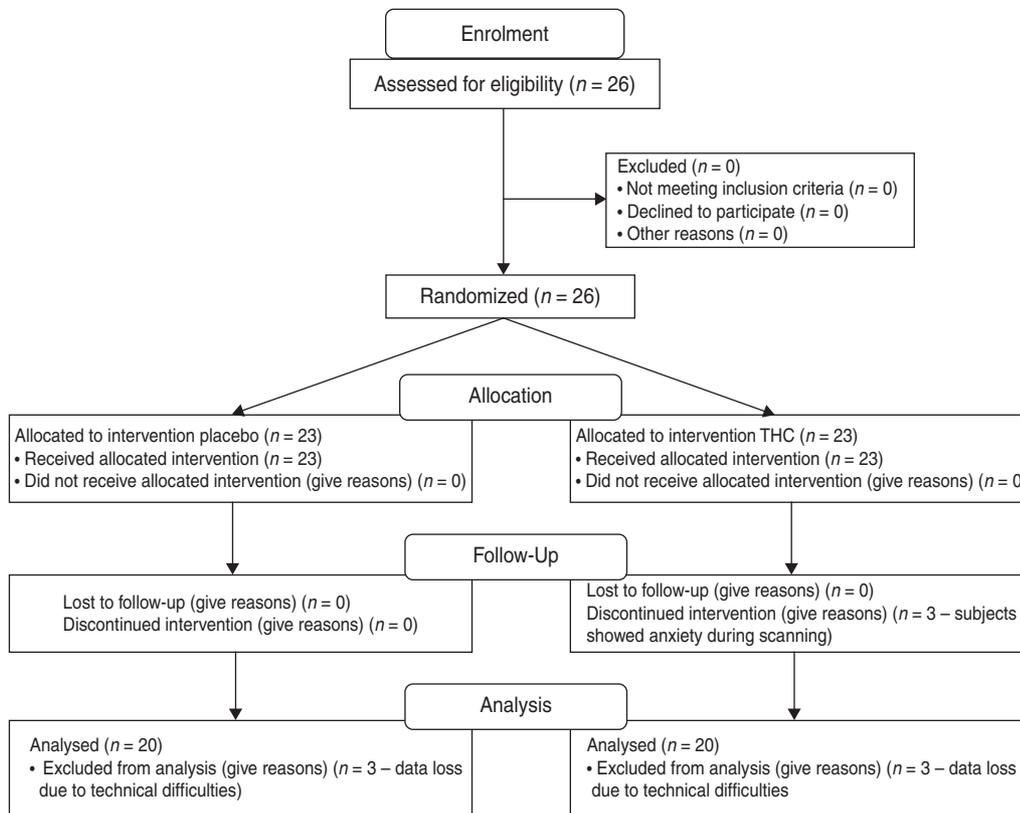


Fig. 1. CONSORT flow diagram illustrating the progress of all subjects in the study. Twenty-six patients were included, three subjects did not finish the study procedures due to feelings of anxiety during scanning, and for both arterial spin labelling and resting-state, data of three subjects were lost due to technical difficulties.

et al. 2011). Here we focus on ASL and resting-state fMRI measurements.

Subjects

Twenty-six male subjects were scanned at the University Medical Centre Utrecht after the inhalation of either placebo or THC, on two separate study days. Inclusion and exclusion criteria are described by van Hell *et al.* (2011), the most important being that subjects needed to be occasional cannabis users (i.e. use at least four times a year but at most once a week) who never had negative experiences after cannabis use (e.g. bad trip, cannabis-induced psychosis). Subjects were excluded if they or their first-degree relatives had been diagnosed with a psychiatric disorder according to DSM-IV criteria. All volunteers gave written informed consent before entry into the study and were paid €250 for participation. For the progress of subjects in the study, see the CONSORT diagram (Fig. 1). The study was approved by the Ethical Committee of the University Medical Centre Utrecht in accordance with the Declaration of Helsinki 2008.

Procedure

At a practice session, subjects completed personality questionnaires and performed the Dutch Adult Reading Test (DART) which estimates verbal intelligence. Subsequently, the procedure of drug administration (inhalation) was practised and participants were familiarized with the scan protocol in a mock scanner to reduce stress effects on the following test days. The actual study consisted of two test days, separated by at least 2 wk to allow for complete clearance of drugs. Subjects were instructed not to use cannabis from 2 wk before the first test day until study completion. Clearance of drugs was tested by means of a urine sample at the beginning of each test day. Additionally, no alcohol was permitted in the 48 h preceding a test day, and subjects needed to refrain from smoking, eating and drinking during the 4 h preceding a test session. A standard breakfast or lunch was provided at the beginning of each test day, to ensure equal states of metabolism on both test days. A catheter was placed in the arm for venous blood sampling.

On test days subjects received THC or placebo by means of a Volcano[®] vaporizer (Zuurman *et al.* 2008) at several time-points. Vehicle (ethanol only) was used as a placebo. The first dose consisted of 6 mg THC or placebo. To maintain equal levels of intoxicating effects throughout the experiment, upload dosages of 1 mg were used, 30 min apart. Two ASL scans were performed, one before and one after the first administration of THC or placebo. Resting-state fMRI was measured after the second or third upload dose of THC or placebo [see Supplementary Fig. S1 (available online), and van Hell *et al.* 2011].

Venous blood samples were collected to determine plasma concentrations of THC and its two most important metabolites, 11-OH-THC and 11-nor-9-carboxy-THC. Blood samples were processed according to Zuurman *et al.* (2008). Subjective effects were measured before and after each task and throughout the test day using self-reported VAS (Bond & Lader, 1974; Bowdle *et al.* 1998; Zuurman *et al.* 2008). Psychedelic effects were assessed using an adapted version of a 13-item VAS, originally described by Bowdle *et al.* (1998). The VAS item 'Feeling high' (defined as the specific psychological effects experienced after THC or cannabis intake) was analysed individually and composite scores of 'External perception' (comprising of six VAS subscores) and 'Internal Perception' (comprising of five VAS subscores) were calculated (see Zuurman *et al.* 2008). Changes in external perception reflect a misperception of an external stimulus or a change in the awareness of the subject's surroundings. Internal perception reflects inner feelings that do not correspond with reality (Zuurman *et al.* 2008). Furthermore, heart rate and respiratory function were monitored continuously during scanning. Heart rate was assessed by measuring the electrocardiogram using four electrodes attached to the subject's chest, and respiratory function was assessed by measuring the expansion of a respiration band around the subject's abdomen. The cardiac and respiratory signals were sampled at a frequency of 500 and 100 Hz, respectively.

Scanning parameters

Image acquisition was performed on a Philips Achieva 3.0-T MR scanner with a Quasar dual gradient set (Philips Medical Systems, The Netherlands).

ASL

Pseudo-continuous labelling was performed by employing a train of radio-frequency (RF) pulses (duration 0.5 ms) with an interpulse pause of 0.5 ms in

combination with a balanced gradient scheme (Dai *et al.* 2008; Wu *et al.* 2007). The duration of labelling was 1650 ms. The control situation was achieved by adding 180° to the phase of every other RF pulse. ASL imaging was performed in combination with background suppression, which consisted of a saturation pulse immediately before labelling and inversion pulses at 1680 ms and 2830 ms after the saturation pulse. Imaging was performed with single-shot echo planar imaging (EPI) in combination with parallel imaging (SENSE factor 2.5). In total, 17 slices of 7-mm thickness were acquired in ascending fashion with an in-plane resolution of 3 × 3 mm². Imaging was performed 1525 ms after labelling stopped. The total scan time for a pair of control and label images was 8 s. For measurement of the magnetization of arterial blood (M_0) and also for segmentation purposes, an inversion recovery sequence was acquired with the same geometry and resolution as the ASL sequence (inversion times: 100–1900 ms with 200 ms intervals, preceded by a saturation pulse at –1680 ms) (van Osch *et al.* 2009).

Resting-state fMRI

For the BOLD resting-state scan, a single run of 400 volumes was obtained over a period of 4 min using a SENSE-PRESTO scan protocol (Neggers *et al.* 2008) (scan parameters: TR 22.5 ms, TE 33.2 ms, flip angle = 10°, FOV 224 × 256 × 160, matrix 56 × 64 × 40, voxel size 4.0 mm isotropic, acquisition time per volume 0.6075 s, 40 slices, sagittal orientation). A high-contrast volume with a flip angle of 27° (FA27) was scanned for registration purposes. Before the functional imaging run, a high-resolution whole-brain anatomical scan was performed (scan parameters: TR 9.4 ms, TE 4.7 ms, flip angle = 8°, FOV 220.8 × 240 × 159.6, matrix 368 × 400 × 113, voxel size 0.6 × 0.6 × 0.6 mm, 266 slices, sagittal orientation).

During the ASL scans as well as the BOLD fMRI resting-state scan, subjects were instructed to lie still and keep their eyes open.

Analysis

Behavioural and physiological measures

VAS feeling high scores were corrected for baseline values and analysed using repeated-measures ANOVA with drug and time as within-subject factors (Zuurman *et al.* 2008). Mean heart rate was calculated for every scan (i.e. ASL before administration, ASL after administration, and BOLD fMRI resting-state, for placebo and THC sessions separately).

ASL

ASL perfusion images were motion-corrected in SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK) prior to subtraction of control images from perfusion-weighted images. The subtraction images were subsequently averaged. Quantitative CBF maps were calculated in ml/100 ml per min from the ASL images using a formula described by van Osch *et al.* (2009).

CBF maps were realigned, normalized, and spatially smoothed (FWHM = 8 mm) in SPM5. Global CBF was calculated for every individual separately, by averaging CBF values in the whole brain excluding cerebrospinal fluid. Individual CBF maps were normalized for global CBF. Group results were analysed using paired-sample *t* tests in SPM5 (placebo *vs.* THC).

Resting-state fMRI

fMRI preprocessing. Resting-state data were preprocessed and analysed using SPM5. Preprocessing of data included realignment of functional images and co-registration with the anatomical scan using the FA27 volume. Subsequently, functional scans were normalized into standard MNI space and smoothed (FWHM = 8 mm).

Before statistical analyses, the BOLD signal was corrected for cardiac and respiratory measures using the RETROICOR method (for details see Glover *et al.* 2000). Cardiac and respiratory phases were calculated per image. A trigger marked peaks in the cardiac signal. Cardiac phase was assumed to advance linearly from 0 to 2π during each peak-to-peak interval and was reset to 0 for the next cycle. For the respiratory phase, both time and amplitude of respiration were accounted for. While inhaling, the phase advanced from 0 to π , and during expiration the phase was negated. A transfer function was used to relate the amplitude of respiration to the phase of respiration (see Glover *et al.* 2000). For each functional image, the cardiac and respiratory phases were calculated. RETROICOR then modelled the relationship between the cardiac and respiratory phases and the BOLD signal, and corrected the BOLD signal accordingly. All further analyses were done on these corrected data.

Temporal signal-to-noise

To assess fluctuations in the resting-state BOLD signal, the tSNR was calculated per voxel by dividing the mean signal of the time-series (400 BOLD fMRI resting-state scans) by the standard deviation (see Supplementary online material for an in-depth explanation of the use of tSNR). For further analysis

Table 1. Demographic characteristics and patterns of drug use

Characteristics	Mean \pm s.d.	Range
Age (yr)	21.1 \pm 2.1	18–27
IQ	105.7 \pm 5.2	94–113
Nicotine use last year (cigarettes/wk)	3.7 \pm 8.4	0–30
Alcohol use last year (units/wk)	16.3 \pm 10.2	2–40
Cannabis use last year (no. occasions)	19.0 \pm 11.2	4–52
Hard-drug use lifetime (no. occasions)	2.3 \pm 3.4	0–15

the tSNR was averaged across all voxels. For comparison between THC and placebo, tSNR values were compared with a paired-sample *t* test in SPM5.

Multiple regression

Multiple regression analysis was used to determine the relationship between imaging data and (psycho)-physiology (heart rate and subjective measures of feeling high). Regions of interest were defined based on a group comparison of THC and placebo maps for the ASL and BOLD data separately, selecting those regions that showed significant differences in perfusion ($T > 3.6$, $p < 0.001$ uncorrected) or resting-state BOLD signal ($T > 3.0$, $p < 0.005$ uncorrected) between THC and placebo conditions. For those regions, difference scores were calculated by subtracting placebo data from THC data.

For the ASL, both heart rate and feeling high scores were used as regression factors, to assess the amount of variance explained. For the BOLD resting-state data, only feeling high scores were used as regression factor, as effects of heart rate had been filtered out during preprocessing (i.e. RETROICOR method).

Results

Demographic characteristics are presented in Table 1. Three subjects did not complete both sessions due to anxiety during scanning and were excluded from the study. Subjects were on average aged 21 ± 2.2 (s.d.) yr and had an IQ of 106 ± 5.2 . Cannabis was used on average on 19 ± 11.2 occasions per year (median 17.5, range 4–52). Subjects smoked on average 3.7 ± 8.4 cigarettes per week (median 0, range 0–30) and 16.3 ± 10.2 units of alcohol were consumed per week (median 14.5, range 2–40). Hard drugs were used on 2.3 ± 3.4 occasions lifetime (median 1.0, range 0–15), and last use was > 6 months prior to participation in the study.

Table 2. Physiological and behavioural effects of placebo and THC

	Placebo	THC	<i>p</i>
Peak plasma concentration THC (ng/ml)	–	84.1 ± 52.6	n.a.
Heart rate (bpm)			
ASL pre-administration	67.6 ± 14.5	69.2 ± 10.3	n.s.
ASL post-administration	67.3 ± 9.8	84.3 ± 23.7	<0.001
BOLD	70.6 ± 20.8	78.0 ± 20.5	n.s.
Feeling High			
ASL pre-administration	0	0	n.a.
ASL post-administration	2.9 ± 10.3	30.3 ± 29.7	<0.01
BOLD	2.1 ± 8.6	24.9 ± 29.2	<0.01

ASL, arterial spin labelling; BOLD, blood oxygen level-dependent; bpm, beats per minute; n.s., non-significant; n.a., not applicable.

Physiological and subjective measures are presented in Table 2. THC plasma concentration reached a maximum of 85.3 ± 52.8 ng/ml 4 min after inhalation of 6 mg THC and decreased rapidly thereafter. Heart rate significantly increased after THC administration compared to placebo ($p < 0.001$) during the post-administration ASL scan. No significant difference in heart rate was found between THC and placebo during the BOLD resting-state scan. Subjective measures of feeling high were significantly increased after THC administration compared to placebo, after both the post-administration ASL scan and the BOLD resting-state scan ($p < 0.01$).

A correlation analysis showed that peak THC plasma concentration (at $t = 4$ min) correlated significantly with peak feeling high after THC (at $t = 27$ min, $r = 0.50$, $p < 0.05$), external perception ($r = 0.47$, $p < 0.05$) and internal perception ($r = 0.52$, $p < 0.05$).

ASL

For three subjects one or more of the pre- or post-administration ASL scans were lost due to technical malfunction of the scanner. Twenty subjects completed the pre- and post-administration ASL scans on both test days and were included in the analyses. For each session pre-administration scans were subtracted from post-administration scans to obtain difference images. A paired-sample *t* test on the difference images (see Fig. 2) revealed that THC increased perfusion compared to placebo in the anterior cingulate cortex, the left superior frontal cortex, and in the left and right insula ($T > 3.6$, $p < 0.001$ uncorrected, cluster size > 10 voxels; see Table 3). THC showed significantly decreased perfusion compared to placebo in the right post-central gyrus, as

well as in the left and right occipital gyrus ($T > 3.6$, $p < 0.001$ uncorrected, cluster size > 10 voxels; see Table 3).

Effects of feeling high and heart rate (both computed as the baseline-corrected values for THC minus placebo) in the regions showing a difference between THC and placebo were assessed by means of regression analysis. Multiple regression analysis showed that the regression model containing these two factors explained a significant part of the variance in perfusion changes (THC *vs.* placebo) in left superior frontal cortex ($r^2 = 0.48$, $p = 0.01$) and the anterior cingulate cortex ($r^2 = 0.35$, $p = 0.04$). In the right occipital gyrus this effect was near-significant ($r^2 = 0.31$, $p = 0.07$). To further break down effects of heart rate and feeling high, regression analyses were performed with either as dependent variable, and all seven regions as independent variables. No effects were observed for heart rate on model fit or separate regressors (regions). Feeling high was significantly explained (model fit $p = 0.005$) and this was mainly due to the left superior frontal cortex ($\beta = -0.70$, $p = 0.002$), and to some extent the left insula ($\beta = 0.42$, $p = 0.045$). Thus, feeling high was correlated strongly negative with superior frontal cortex, and moderately positive with left anterior insula. Finally, regions were compared directly to one another, revealing only a positive correlation between the anterior cingulate and the right insula ($r = 0.704$, $p = 0.001$).

Resting-state fMRI

For three subjects one of the resting-state scans was lost due to technical problems. Twenty subjects completed the resting-state scan on both test days and were included in the analysis.

Table 3. Areas in which THC and placebo show significant differences in perfusion ($T > 3.6$, $p < 0.001$ uncorrected, cluster size > 10 voxels)

Area	Cluster size (no. of voxels)	Peak t value	p value cluster level (uncorrected)	Location of t value		
				x	y	z
THC > placebo						
Anterior cingulate cortex	16	6.31	0.007	-3	42	28
Superior frontal cortex L	12	5.56	0.017	-33	63	0
Insula L	59	4.92	0.000	-51	21	7
Insula R	16	4.24	0.009	30	24	-7
Placebo > THC						
Post-central gyrus R	11	5.63	0.021	63	-3	21
Occipital gyrus R	50	5.97	0.000	42	-78	0
Occipital gyrus L	12	5.30	0.017	-36	-87	0

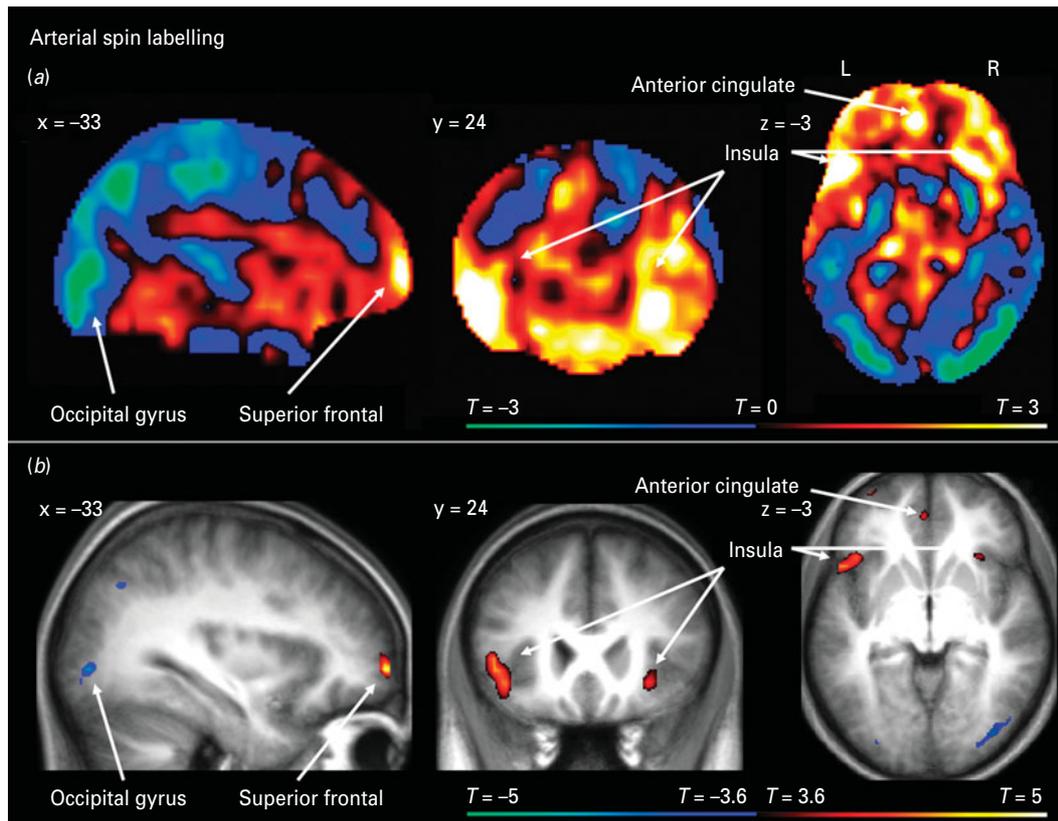


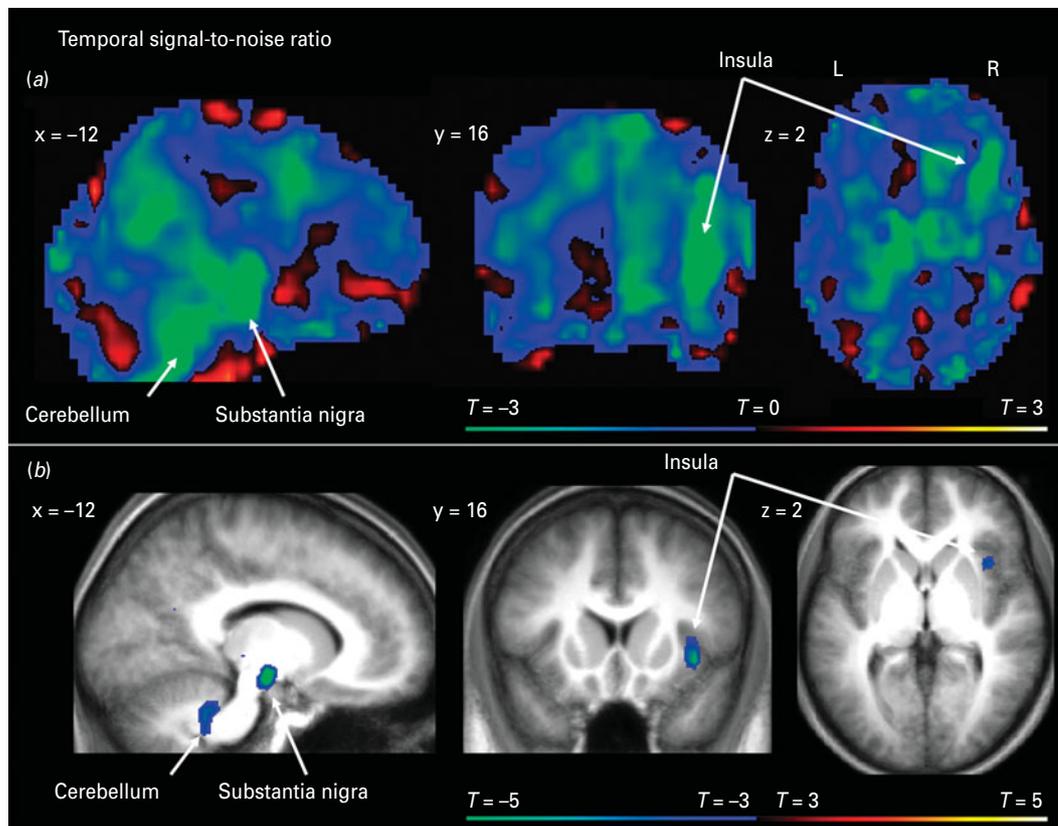
Fig. 2. Arterial-spin-labelling difference maps between THC and placebo sessions. Red/yellow indicates areas showing increased perfusion during THC compared to placebo, and blue/green indicates areas showing increased perfusion during placebo compared to THC. (a) Overall differences between THC and placebo. (b) Areas that are significantly different between THC and placebo ($T > 3.6$, $p < 0.001$ uncorrected, cluster size > 10 voxels). L, Left; R, right.

Signal fluctuations in the BOLD resting-state data were assessed by comparing the tSNR images between THC and placebo with a paired-sample t test

(see Fig. 3). THC reduced tSNR in the right insula, the left cerebellum, and the left substantia nigra ($T > 3.0$, $p < 0.005$ uncorrected, cluster size > 10 voxels;

Table 4. Areas in which THC and placebo show significant differences in temporal signal-to-noise ratio ($T > 3.0$, $p < 0.005$ uncorrected, cluster size > 10 voxels)

Area	Cluster size (no. of voxels)	Peak t value	p value cluster level (uncorrected)	Location of t value		
				x	y	z
Placebo > THC						
Substantia nigra L	16	5.72	0.022	-12	-12	-12
Insula R	19	4.59	0.014	40	16	-4
Cerebellum L	15	3.78	0.026	-12	-44	-32

**Fig. 3.** Fluctuations in the fMRI BOLD signal; difference maps of signal-to-noise ratio (tSNR) between THC and placebo sessions. Red/yellow indicates areas showing higher tSNR during THC than during placebo, and blue/green indicates areas showing higher tSNR during placebo than during THC. (a) Overall differences between THC and placebo. (b) Areas that are significantly different between THC and placebo ($T > 3$, $p < 0.005$ uncorrected, cluster size > 10 voxels). L, Left; R, right.

see Table 4) compared to placebo. No areas showed significantly higher tSNR after THC administration compared to placebo. This indicates that BOLD fMRI signal fluctuations increased after THC administration. Multiple regression analysis showed no significant correlation between THC effects on tSNR and feeling high scores. Comparing THC effects in the

ASL and the tSNR regions, only a trend for a negative correlation was found in the insula (ASL insula vs. tSNR right insula, $r = -0.45$, $p = 0.06$). Further, tSNR values were assessed in the regions where ASL showed differences between THC and placebo. In these regions, tSNR values were similar for THC and placebo.

Discussion

This study investigated the acute effects of THC on resting-state brain perfusion and activity, measured with ASL and BOLD fMRI, respectively, in association with the psychotropic effect (feeling high). Compared to placebo, THC increased perfusion in the anterior cingulate cortex, the superior frontal cortex, and the insula, whereas perfusion was decreased in the post-central gyrus and the occipital gyrus. The feeling high effect of THC was negatively correlated with changes in perfusion in the left superior frontal cortex. THC increased fluctuation of the resting-state BOLD signal in the insula, the substantia nigra and the cerebellum, reflecting increased baseline activity.

Effects of THC were observed on both perfusion and on resting-state signal fluctuation in one specific region, the right anterior insula. This is a structure integrating visceral, autonomic and hedonic information (Uddin & Menon, 2009), which is represented in its interconnections with the amygdala, hypothalamus, anterior cingulate cortex and orbitofrontal cortex (Craig, 2009a). The insula is implicated in many cognitive brain functions, such as emotion, attention, and motivation, and has recently been shown to play an important role in addiction (Naqvi & Bechara, 2009). Insular activity is associated with interoceptive awareness, a conscious representation of self (Critchley *et al.* 2004) and self-reflection (Modinos *et al.* 2009), essential for generating a mental image of one's physical state (Craig, 2002). Moreover, the insula is regarded as the primary sensory cortex for inner body feelings like hunger and thirst, autonomic processes and awareness thereof (Craig, 2008). A recent report on epileptic seizures originating in the insula indicated that this brain region plays an important role in emotion and bodily feelings in relation to subjective awareness. It was observed that increased insular activity due to epileptic activity was associated with feelings of intense wellbeing (Picard & Craig, 2009). Hence, our finding of increased perfusion and elevated signal fluctuation in the insula after THC administration could signify an increase in interoceptive awareness, as well as an increase in wellbeing and/or euphoria. The moderate correlation between feeling high and perfusion in (left) anterior insula supports this notion. Interestingly, perfusion was decreased by THC in somatosensory cortex, and in visual cortex which has been reported before (O'Leary *et al.* 2007). It is not directly clear how this related to the psychotropic effects of THC, but it is tempting to associate it with the typically reported altered sensory perception

(Green *et al.* 2003). However, this needs further investigation.

The anterior cingulate cortex, associated with various functions including attentional processes and emotional control (Bush *et al.* 2000; Critchley, 2005), evidenced elevated perfusion after THC administration which was correlated with that in right insula. These two regions are highly interconnected and are often activated together during emotional processing or bodily awareness (Craig, 2002, 2009b). Taylor *et al.* (2009) showed strong resting-state functional connectivity between these regions and proposed that this may reflect integration of interoceptive information with emotional salience to form a subjective image of the bodily state. Craig (2009a, b) proposed a model stating that the (anterior) insula is more implicated in the sensory aspects of emotional processing, while the anterior cingulate cortex is part of the motor limbic cortices that constitutes motivation and initiates behaviour. Given that acute effects of THC include changes in bodily awareness, in motivational drive and in emotionality, our finding of correlated perfusion increases in these two regions suggests increased activity commensurate with the model of Craig, extending it with the concept of a related psychotropic effect of THC.

A strong increase in left superior frontal cortex perfusion following THC is associated with a relatively low rating of feeling high, and vice versa. This region matches the 'lateral rostral prefrontal cortex' described as being connected to anterior cingulate and anterior insula (Gilbert *et al.* 2010). Given the notion that this network is primarily involved in tasks that require cognitive control (Bush *et al.* 2000; Gilbert *et al.* 2010), our data suggest that the superior frontal cortex may serve to *suppress* feeling high. Activity in this region may then reflect an individual's ability to resist or overcome the sensory and perceptual effects in the service of maintaining control over one's mental state.

In literature, subjective measures of intoxication and of depersonalization due to THC are reportedly associated with magnitude of CBF increase in frontal regions (Mathew *et al.* 1997, 1999). Whether these disagree with our findings is not clear, because in those studies subjects rated 'intoxication' as opposed to 'feeling high'. Intoxication covers a mix of components including the feeling of being in a different mental state, euphoria, anxiety, drowsiness and changes in bodily awareness. Zuurman *et al.* (2009) recently reviewed the literature that assesses acute effects of cannabis or THC, and indicated that the most reliable and stable effects that are found after acute THC or cannabis administration are elevated heart

rate and subjective effects (i.e. feeling high, stoned or euphoric). In addition, they differentiate between the scales 'high' and 'drug effect', where 'drug effect' represents 'strength of drug effect, feelings of intoxication, or subjective psychological effects'. The scales assessing 'drug effect' were not as sensitive as the scales for 'high', and addressed subjective changes that are less specific for THC or cannabis.

Differences in designs of the studies (e.g. between-subject comparisons as opposed to within-subject comparisons in the current study) or in the methods used (PET *vs.* ASL) may also contribute to the apparent discrepancy with the present study. In the present study we corrected for changes in heart rate in the ASL group analysis and in preprocessing of the resting-state data. Although in the PET studies corrections are applied for global CBF, regional effects of heart rate may not be fully corrected for. The general effect of THC in the present study (elevated perfusion in frontal regions) is, however, in line with previous PET studies (Chang & Chronicle, 2007; Martin-Santos *et al.* 2010; Quickfall & Crockford, 2006). Nevertheless, the observed alterations in brain neurophysiology during rest may reflect physical and emotional sensations (euphoria, feeling high) that form the psychotropic effects of THC.

One would expect higher CBF to increase resting-state fluctuations. However, direct comparison between baseline perfusion measures and resting-state tSNR showed that CBF may not simply amplify resting-state fluctuations, as tSNR values were strikingly similar for THC and placebo in the regions where ASL showed differences between the two drugs. This is worth further investigation with direct manipulations of CBF in a separate study.

THC increased signal fluctuation in the right insula, substantia nigra, and cerebellum. As these regions also exhibit high densities of cannabinoid receptors (Ameri, 1999; Iversen, 2003), it is plausible that THC directly affects neuronal activity by influencing endocannabinoid signalling in these regions, rather than indirectly affecting neuronal activity by changing neurotransmission through cannabinoid alteration of dopaminergic or glutamatergic signalling. The elevation of signal fluctuation is then most likely caused by increased spontaneous activity or by increased amplitude of low frequencies (which dominate our measure of signal fluctuation). This is supported by the fact that the cannabinoid system has a neuromodulatory role in the central nervous system and is capable of modulating regional synaptic activity (Piomelli, 2003; Pope *et al.* 2010). Interestingly, the feeling high effect of THC is not affected by the dopamine antagonist

haloperidol, supporting the notion of a direct endocannabinoid basis (D'Souza *et al.* 2008; Liem-Moolenaar *et al.* 2010).

The present study has several limitations. For one, the effects were not overly strong, so a rather liberal threshold was used when comparing THC and placebo in the imaging analyses. It may be that correction for pre-administration limited sensitivity for changes. Further, the resting-state fMRI scan was made during a relatively short period of time (i.e. 4 min), where most studies use at least 8 min of rest (Damoiseaux *et al.* 2006; van den Heuvel *et al.* 2008). The tight THC administration scheme (van Hell *et al.* 2011) did not allow for a longer scan between upload doses. Thus, more regions may be involved in the psychotropic effects of THC than what is reported here. The ASL scans did not cover the cerebellum, perfusion of which is likely to be affected by THC given the findings in earlier PET studies (Martin-Santos *et al.* 2010).

Although the study was designed to be double blind, THC induced behavioural effects that were identified by most subjects, possibly causing expectancy effects across sessions. We tried to minimize the influence of expectancy by using a randomized cross-over design. All subjects received THC and placebo on two separate sessions. By randomizing the order of administration between subjects (50% of the subjects receive THC first, 50% placebo first), expectancy effects were balanced across sessions. Further, even though most subjects identified the administered drug correctly, some subjects did not notice on which test day they had received THC. Still, we cannot exclude that expectancy effects may have affected our results to some extent.

Taken together, our findings indicate that THC increases perfusion and signal fluctuations in anterior insula. Given the functions attributed to the anterior insula, we postulate that it plays an important role in the effects of THC that lead up to feeling high, which may be related to enhanced interoceptive awareness. Engagement of the left prefrontal cortex could serve to suppress this effect. Given the reported role of the prefrontal cortex in activities that require top-down control over cognitive processes (Gilbert *et al.* 2010), it may well be that engagement of this region enables one to resist the subjective effects of THC, including feeling high.

Our findings also indicate usefulness of baseline measurements in phMRI studies. THC-induced changes in fMRI brain activity during a cognitive challenge may not solely be cognitive in nature, as THC exerts subjective and physiological effects that can interact with cognitive and emotional brain function. In

conclusion, the findings raise the possibility that the primary psychotropic effect of THC, i.e. feeling high, results from an interplay of regions involved in subjective interoceptive awareness and in cognitive control.

Note

Supplementary material accompanies this paper on the Journal's website (<http://journals.cambridge.org/pnp>).

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Statement of Interest

None.

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